

WHAT IS CLAIMED IS:

1. A polynucleotide encoding a fusion protein capable of being expressed in a plant or a plant cell, comprising:

- 5 (a) a coding region that encodes said fusion protein and includes:
- i. a plant viral coat protein from a single-stranded plus-sense RNA virus, and fused thereto,
 - ii. a peptide comprising amino acids MGSDGAVQPDGGQPAV (SEQ ID NO:1) or a fragment thereof;
- 10 and
- (b) a promoter functional in plants that is 5' to the coding region.

2. A polynucleotide according to claim 1, wherein the peptide is fused to the N-terminus of the plant viral coat protein.

3. A polynucleotide according to claim 1, wherein the peptide is fused to the C-terminus of the plant viral coat protein.

4. A polynucleotide according to claim 1, wherein said fusion protein is an internal fusion protein with respect to the coat protein.

5. A polynucleotide according to claim 1, further comprising
- (c) a fusion joint having a leaky stop codon from a single-stranded plus-sense RNA virus.

6. A polynucleotide according to claim 1, wherein the peptide MGSDGAVQPDGGQPAV or fragment comprises an antigen.

7. A polynucleotide according to claim 1, wherein the coat protein is a tobacco mosaic virus coat protein.

8. A polynucleotide according to claim 1 wherein the coat protein is a tobamovirus coat protein.

5 9. A recombinant plant viral genome comprising a polynucleotide according to claim 1.

10. A recombinant plant virus particle, comprising a genome according to claim 9.

10 11. A recombinant plant virus having a coat protein encoded by a polynucleotide according to claim 1.

12. A plant cell comprising a polynucleotide according to claim 1.

15 13. A plant cell comprising a recombinant plant viral genome according to claim 9.

14. A plant cell comprising a recombinant plant virus particle according to claim 10.

20 15. A plant cell comprising a recombinant plant virus according to claim 11.

16. A plant comprising a polynucleotide according to claim 1.

25 17. A plant comprising a recombinant plant viral genome according to claim 9.

18. A plant comprising a recombinant plant virus particle according to claim 10.

30 19. A plant comprising a recombinant plant virus according to claim 11.

20. A polynucleotide encoding a fusion protein capable of being expressed in a plant or a plant cell, comprising:

- (a) a coding region that encodes said fusion protein and includes:
- i. a plant viral coat protein from a single-stranded plus-sense RNA virus, and fused thereto,
 - ii. a peptide comprising amino acids MGQPDGGQPAVRNERAT (SEQ ID NO 2) or a fragment thereof;
- and
- (b) a promoter functional in plants that is 5' to the coding region.

21. A polynucleotide according to claim 20, wherein the peptide is fused to the N-terminus of the plant viral coat protein.

22. A polynucleotide according to claim 20, wherein the peptide is fused to the C-terminus of the plant viral coat protein.

23. A polynucleotide according to claim 20, wherein said fusion protein is an internal fusion protein with respect to the coat protein.

24. A polynucleotide according to claim 20, further comprising

(c) a fusion joint having a leaky stop codon from a single-stranded plus-sense RNA virus.

25. A polynucleotide according to claim 20, wherein the peptide MGQPDGGQPAVRNERAT or a fragment comprises an antigen.

26. A polynucleotide according to claim 20, wherein the coat protein is a tobacco mosaic virus coat protein.

27. A polynucleotide according to claim 20 wherein the coat protein is a tobamovirus coat protein.

28. A recombinant plant viral genome comprising a polynucleotide according to claim 20.

29. A recombinant plant virus particle, comprising a genome according to claim 28.

30. A recombinant plant virus, wherein the coat protein is encoded by a polynucleotide according to claim 20.

31. A plant cell comprising a polynucleotide according to claim 20.

32. A plant cell comprising a recombinant plant viral genome according to claim 28.

33. A plant cell comprising a recombinant plant virus particle according to claim 29.

34. A plant cell comprising a recombinant plant virus according to claim 30.

35. A plant comprising a polynucleotide according to claim 20.

36. A plant comprising a recombinant plant viral genome according to claim 28.

37. A plant comprising a recombinant plant virus particle according to claim 29.

38. A plant comprising a recombinant plant virus according to claim 30.

39. An immunochemical reagent comprising a fusion protein capable of being produced in a plant or a plant cell, wherein the fusion protein comprises

- (i) a plant viral coat protein from a single-stranded plus-sense RNA virus; and
- (ii) a peptide comprising amino acids MGQPDGGQPAVRNERAT or a fragment thereof fused to the N-terminus of the coat protein.

40. A vaccine for the protection of mammals against parvovirus comprising the immunochemical reagent of claim 39.

41. A vaccine according to claim 40 together with a pharmaceutically or veterinarily acceptable carrier or excipient.

42. An immunochemical reagent comprising a recombinant plant virus, wherein at least one capsid of the coat protein is a fusion protein capable of being produced in a plant or a plant cell, wherein the fusion protein comprises

- (i) a plant viral coat protein from a single-stranded plus-sense RNA virus; and
- (ii) a peptide comprising amino acids MGSDGAVQPDGGQPAV or a fragment thereof fused to the N-terminus of the coat protein.

43. A vaccine for the protection of mammals against parvovirus comprising an immunochemical reagent according to claim 42.

44. A vaccine according to claim 43 together with a pharmaceutically or veterinarily acceptable carrier.

45. A vaccine according to claim 40, wherein the fusion protein is in a recombinant plant virus.

46. A vaccine according to claim 45 wherein said virus is a live virus.

47. A vaccine according to claim 43, wherein the recombinant plant virus is a live virus.

48. A vaccine composition comprising a live recombinant plant virus according to claim 46 and a pharmaceutically or veterinarily acceptable carrier or excipient.

49. A vaccine composition comprising a live recombinant plant virus according to claim 47 and a pharmaceutically or veterinarily acceptable carrier or excipient.

50. A method of making the polynucleotide of claim 1, comprising ligating an oligonucleotide encoding a peptide having the sequence MGSDGAVQPDGGQPAV or a fragment thereof to a viral coat protein gene.

51. A method of making a recombinant plant viral genome comprising (a) inserting an oligonucleotide encoding a peptide having the sequence MGSDGAVQPDGGQPAV or a fragment thereof into the genome of a single-stranded plus-sense RNA virus so that said oligonucleotide is fused in frame with a plant viral coat protein gene and under the control of a promoter functional in plants; or

(b) ligating the polynucleotide of claim 1 to the genome of a single-stranded plus-sense RNA virus, thereby making said recombinant plant viral genome.

52. A method of making the recombinant plant virus that encodes a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGSDGAVQPDGGQPAV or a fragment thereof, comprising the steps of:

- 5
- (a) ligating DNA encoding the fusion protein which protein is capable of being expressed in a plant or a plant cell, to a DNA copy of the genome of a single-stranded plus-sense RNA virus;
 - (b) transcribing said ligated DNA to RNA; and
 - (c) infecting a host plant or plant cell with said transcribed RNA,
- so that said plant or plant cell makes said recombinant plant virus.

10 53. A method of making the recombinant plant virus that encodes a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGSDGAVQPDGGQPAV or a fragment thereof, comprising the steps of;

- 15
- (a) ligating DNA encoding the fusion protein which protein is capable of being expressed in a plant or a plant cell, to a DNA copy of the genome of a single-stranded plus-sense RNA virus, under the control of a promoter that is functional in plants; and
 - (b) transforming or transfecting a host plant or plant cell with said ligated DNA, so that said DNA is expressed in said plant or plant cell,
- whereby said plant or plant cell makes said recombinant plant virus.

20 54. A method of making a plant cell that produces a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGSDGAVQPDGGQPAV or a fragment thereof, comprising transforming or transfecting a host plant cell with the plant viral genome of claim 51.

25 55. A method of making a plant cell that produces a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGSDGAVQPDGGQPAV or a fragment thereof, comprising infecting a host plant cell with the plant virus of claim 52.

30

56. A method of making a plant cell that produces a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGSDGAVQPDGGQPAV or a fragment thereof, comprising infecting a host plant cell with the plant virus of claim 53.

57. A method of making a plant that produces a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGSDGAVQPDGGQPAV or a fragment thereof, comprising transforming or transfecting a host plant with the plant viral genome of claim 51.

58. A method of making a plant that produces a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGSDGAVQPDGGQPAV or a fragment thereof, comprising infecting a host plant with the plant virus of claim 52.

59. A method of making a plant that produces a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGSDGAVQPDGGQPAV or a fragment thereof, comprising infecting a host plant with the plant virus of claim 53.

60. A method of making the polynucleotide of claim 20, comprising ligating an oligonucleotide encoding a peptide having the sequence MGQPDGGQPAVRNERAT or a fragment thereof to a viral coat protein gene.

61. A method of making a recombinant plant viral genome comprising
(a) inserting an oligonucleotide encoding a peptide having the sequence MGQPDGGQPAVRNERAT or a fragment thereof into the genome of a single-stranded plus-sense RNA virus so that said oligonucleotide is fused in frame with a plant viral coat protein gene and under the control of a promoter functional in plants; or

(b) ligating the polynucleotide of claim 1 to the genome of a single-stranded plus-sense RNA virus thereby making said recombinant plant viral genome.

5 62. A method of making the recombinant plant virus that encodes a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGQPDGGQPAVRNERAT or a fragment thereof, comprising the steps of;

- 10 (a) ligating DNA encoding the fusion protein which protein is capable of being expressed in a plant or a plant cell, to a DNA copy of the genome of a single-stranded plus-sense RNA virus;
- (b) transcribing said ligated DNA to RNA; and
- (c) infecting a host plant or plant cell with said transcribed RNA, so that said plant or plant cell makes said recombinant plant virus.

15 63. A method of making the recombinant plant virus that encodes a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGQPDGGQPAVRNERAT or a fragment thereof, comprising the steps of;

- 20 (a) ligating DNA encoding the fusion protein which protein is capable of being expressed in a plant or a plant cell, to a DNA copy of the genome of a single-stranded plus-sense RNA virus, under the control of a promoter that is functional in plants; and
- (b) transforming or transfecting a host plant or plant cell with said ligated DNA, so that said DNA is expressed in said plant or plant cell, whereby said plant or plant cell makes said recombinant plant virus.
- 25

64. A method of making a plant cell that produces a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGQPDGGQPAVRNERAT or a fragment thereof, comprising transforming or transfecting a host plant cell with the plant viral genome of claim 61.

65. A method of making a plant cell that produces a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGQPDGGQPAVRNERAT or a fragment thereof, comprising infecting a host plant cell with the plant virus of claim 62.

66. A method of making a plant cell that produces a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGQPDGGQPAVRNERAT or a fragment thereof, comprising infecting a host plant cell with the plant virus of claim 63.

67. A method of making a plant that produces a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGQPDGGQPAVRNERAT or a fragment thereof, comprising transforming or transfecting a host plant with the plant viral genome of claim 61.

68. A method of making a plant that produces a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGQPDGGQPAVRNERAT or a fragment thereof, comprising infecting a host plant with the plant virus of claim 62.

69. A method of making a plant that produces a fusion protein that includes a plant viral coat protein from a single-stranded plus-sense RNA virus and a peptide comprising amino acids MGQPDGGQPAVRNERAT or a fragment thereof, comprising infecting a host plant with the plant virus of claim 63.

70. A method of isolating a virus, comprising:

- (a) Homogenizing virus-containing plant tissue in $\text{Na}_2\text{S}_2\text{O}_5$;
- (b) Straining the homogenate to obtain green juice;
- (c) Adjusting the pH of the green juice to 5.0 with acid;
- (d) Heating the green juice to about 47°C for a period of about 5 minutes followed by cooling to about 5°C ;
- (e) Centrifuging the green juice at about $6000 \times g$ for about 3 minutes to obtain a supernatant and pellet;
- (f) Precipitating the supernatant in polyethylene glycol and NaCl to obtain a precipitate;
- (g) Resuspending the precipitate in water at a concentration of about 1 mg per ml;
- (h) Extracting the precipitate in chloroform and butanol and centrifuging the extract
- (i) Recovering and lyophilizing the aqueous phase of the centrifuged material;
- (j) Resuspending the lyophilized material at a concentration of about 5 to about 10 mg per ml water.

71. A method of isolating a virus, comprising:

- (a) Grinding virus-containing plant material in a buffer to obtain a homogenate;
- (b) Straining the homogenate to obtain green juice, and adding thereto polyethyleneimine to a concentration of about 0.1% (v/v)
- (c) Stirring at about 4°C for about 30 minutes followed by centrifuging ; at about $3000 \times g$ for about 5 minutes to obtain a supernatant;
- (d) Precipitating the supernatant in polyethylene glycol and NaCl to obtain a precipitate;
- (e) Resuspending the precipitate in water at a concentration of about 1 mg per ml;

- (f) Extracting the resuspended material in chloroform and butanol and centrifuging the extracted material;
- (g) Recovering and lyophilizing the aqueous phase; and
- (h) Resuspending the lyophilized material at a concentration of about 5 to about 10 mg per ml water.

5

ALLAI

ALLAI